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(54) Title: METAL SUBSTITUTED NON CENTROSIMMETRICAL PHTHALOCYANINE ANALOGUES, THEIR PREPARATION AND USE IN PHOTODYNAMIC THERAPY AND IN VIVO DIAGNOSTIC

(57) Abstract: Phthalocyanine analogues having an active group able to link the phthalocyanine to carriers molecules and phthalocyanine analogues as phthalocyanine-carrier conjugates showing enhanced photodynamic properties, red shifted absorption characteristic, all useful for photodynamic therapy, are described. Photosensitizers themselves or the photosensitizers-carrier conjugates are useful compounds either for treatment of various infectious diseases, the in vivo eradication of micro-organisms as well as diseases characterized by cellular hyperproliferation, in particular tumours psoriasis, actinic keratosis, atheromas, endoarterial hyperplasia and prostate hyperplasia. The above compounds can be also useful for blood and blood derivatives sterilization and as in vivo/vitro diagnostics.

METAL SUBSTITUTED NON CENTROSIMMETRICAL PHTHALOCYANINE ANALOGUES, THEIR PREPARATION AND USE IN PHOTODYNAMIC THERAPY AND *IN VIVO* DIAGNOSTIC.

Field of the invention

The invention relates to non centrosimmetrical Me-phthalocyanine analogues having a site specific reactive group able to link them to biological carriers through covalent bonds, good solubility and enhanced photodynamic properties.

The invention refers also to conjugates consisting of metal-phthalocyanine analogues as above defined and aminoacids, polypeptides, proteins, antibodies, polysaccharides and aptamers.

Moreover the invention refers to processes for preparing the above said products and to pharmaceutical compositions containing them, useful for *in vivo/ex vivo* therapeutic treatment and *in vitro/in vivo* diagnostic purposes.

State of the Art

It is known that organic molecules able to produce singlet oxygen, as a result of light irradiation, may have photoenhanced biocidal activity. The biocidal properties of such molecules, occurring with essentially every living form, make these molecules extremely interesting for therapeutic applications.

The practical application of photosensitizers is generally limited due to the fact that those products are either allergens, in some cases are retained in human skin or non specifically localized in ill tissues, thus leading to phototoxicity after light exposition.

Early work in the 1970's, followed from studies in the 1980's, have shown that photosensitizers can be used against viruses, fungi, bacteria and eukariote cells.

Dougherty et al. (Cancer Res., 1978, 38, 262) has pioneered the field of PDT for tumour treatment with photoactivatable dyes in association with long wavelength radiation.

Even if a great deal of advancements in this field have been performed, there is still a need for new compounds to be used both in PDT therapy of infectious diseases and tumour conditions.

Deficiencies of earlier agents, based on naturally-occurring starting materials, can be overcome by using synthetic chemically pure photoactivatable products, more

readily prone to further chemical structural modifications.

Among the new various photosensitizers, so called "second generation photosensitizers", worth of further development, phthalocyanines, short name of tetrabenzotetraaza porphyrins, appear ones of the most important photosensitizers for therapeutic applications.

Zn(II)-phthalocyanines having applications in photodynamic therapy (PDT) and diagnosis are described in EP 906 758, in the name of the same Applicant.

The therein described products show very interesting properties, in fact can be easily prepared, have a low intrinsic toxicity (dark toxicity), while they are active as photosensitizers for singlet oxygen production or radicals, are selectively up-taken in proliferating cells, rapidly degraded and eliminated from non target tissues after administration and finally, are available as chemically pure and stable compounds, eventually prone to further synthetic modification in order to be more selective.

In order to further improve the therapeutic potential of these photosensitizers, they are specifically designed in order to be easily linked to carriers able by themselves to specifically recognize biological targets, thus providing the way to reach the living form to be eradicated, while not affecting the surrounding healthy cells.

In particular, in order to allow chemical conjugation to biological relevant macromolecular carriers, the phthalocyanines should bear reactive functions, specific for only one functional group of the macromolecule and hydrophilic groups, with the aim of not introducing variation in the overall hydrophilic nature of the conjugate. Moreover the presence of suitable substituents should not interfere with the photodynamic properties of phthalocyanine.

It must be noted that previously described phthalocyanines are not suitable for linking to biologically relevant carriers because they lack specific reactive groups, therefore no reaction can occur unless cross linking reagents especially devised are used, or they have more than one reactive groups and therefore their use in the conjugation procedures results in an uncontrolled proteins polymerization and crosslinking which in turn, leads to difficulty to purify and analyse conjugates preparation.

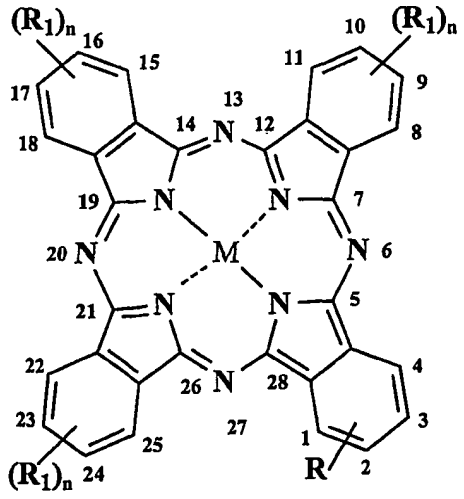
Organic solvents soluble, hydrophobic phthalocyanines bearing one reactive group have been previously described (H. Kliesh et al. Liebigs Ann. 1995,1269-1273),

however the overall hydrophobic properties of these phthalocyanines cause a modification of the macromolecular hydrophilic balance, have a negative effect on the conjugate stability, may cause irreversible aggregation problems and lead to carriers denaturation.

In view of the above said, it is essential to provide new products having both physical-chemical and photodynamic enhanced properties, thus allowing their use against a wider spectrum of pathologies, while lowering the undesired side effects that were both accomplished with the products described in this invention.

Summary of the invention

The present invention refers to Me-phthalocyanines analogues of formula (I)



(I)

wherein :

n is 1, 2 or 4;

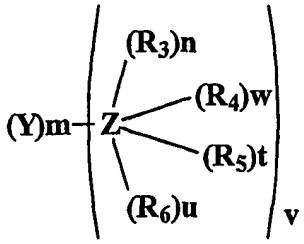
M is chosen in the group consisting of Zn, $Si(OR_7)_2$ and $AlOR_7$ wherein R_7 is chosen in the group consisting of H, C_{1-15} alkyl and pharmaceutically acceptable salts thereof;

R is chosen in the groups consisting of: -COOH, -SH, -OH, $-NH_2$, $-CO-CH_2-Br$, $-SO_2Cl$, maleimide, hydrazide, phenol, imidate, biotine, possibly bound to the phthalocyanine nucleus through a suitable linker and

R_1 is H or, when n = 2 and the two groups R_1 are in the positions 9,10,16,17,23,24, said two groups R_1 can form a saturated or unsaturated heterocycle, possibly substituted, which may contain up to two heteroatoms

chosen from N,O, S; or R_1 is represented by the group $(X)_pR_2$, wherein:

X is chosen in the group consisting of O, S, $-NR_5$ and $-CH_2-$ and R_2 is



where :

Y is chosen in the group consisting of C_{1-10} alkyl and phenyl, possibly substituted, or it forms with the Z group, to which it is bound, a saturated or unsaturated heterocycle, possibly substituted, which may contain up to two heteroatoms chosen in the group consisting of N, O and S;

Z is chosen in the group consisting of -N, $-CH_2N$ and $-CONHCH_2CH_2N$;

R_3 and R_4, equal or different from one another, are chosen in the group consisting of C_{1-15} alkyl and phenyl, or form with the Z group, to which they are bound, a saturated or unsaturated heterocycle, possibly substituted, which may contain up to two heteroatoms chosen in the group consisting of N, O and S;

R_5 and R_6, equal or different from one another, are chosen in the group consisting of H and C_{1-15} alkyl ;

m, n, p, w, t and u, independently from one another, are 0 or 1; and

v is an integer comprised between 1 and 3;

with the proviso that:

R is in the position 1 or 2.

R_1 is in the positions: 8(11), 15(18), 22(25), or 9(10), 16(17), 23(24) when n = 1.

R_1 is in the positions: 8,11,15,18, 22,25 or 9,10,16,17,23,24 when n = 2.

The invention refers also to the compounds of formula (I) as above defined conjugated with bio-organic carriers such as aminoacids, polypeptides, proteins polysaccharides and aptamers.

Brief description of the drawing

Figure 1 represents the variation of the colony forming units (CFU) of *C. albicans* vs. the concentration (μM) of the compounds according to Examples 7 and 8, indicated in the figure as MRLP 090 and MRLP 091 respectively.

Detailed description of the invention

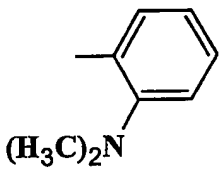
The present invention allows to overcome the above said problem thanks to the compounds of formula (I) as above defined.

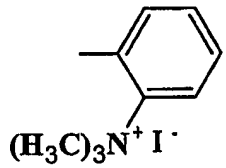
According to the present invention Zn(II)-phthalocyanines are preferred wherein R is as previously defined, and R_1 is represented by the group $(X)_pR_2$, previously defined.

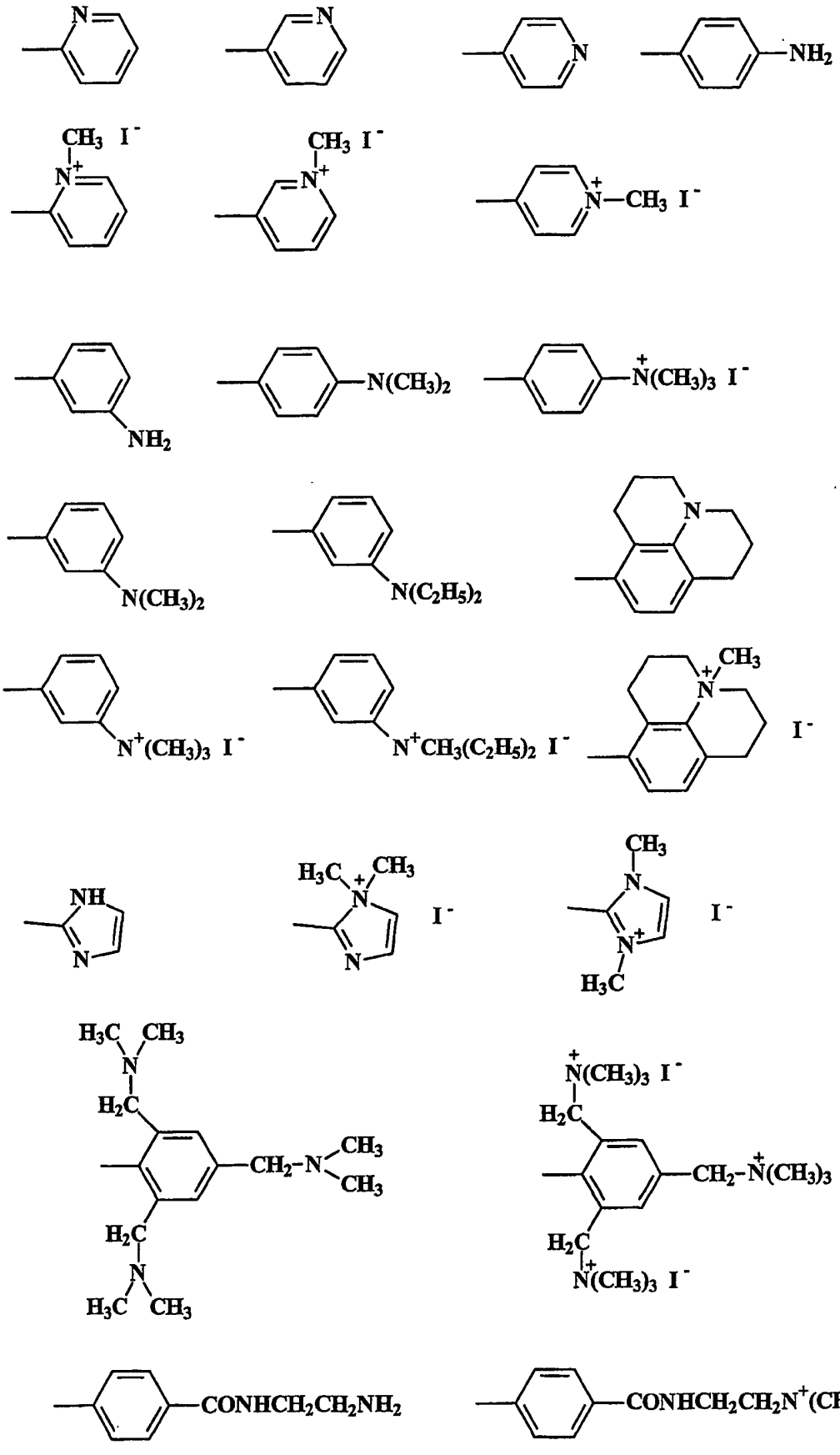
According to the invention, the definition "suitable linker" it is intended in the sense commonly given to this definition in the field of protein and nucleic acid modification (S. S. Wang, Chemistry of Protein Conjugation and Cross-Linking CRC Press, Inc. 1993, G.T. Hermanson Bioconjugate Techniques Academic Press, 1996) i.e. an aliphatic or aromatic moiety which act as a spacer between the phthalocyanine nucleus and the biological macromolecules, in order to satisfy wanted sterical and/or structural requirements.

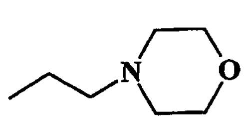
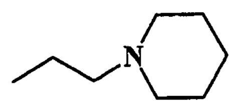
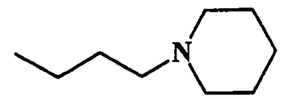
By saturated or unsaturated heterocycle possibly substituted, as defined in the above general formula, the following are preferably meant : morpholine, piperidine, pyridine, pyrimidine, piperazine, pyrrolidine, pyrroline, imidazole, aniline, and julolidine (2,3,6,7-tetrahydro-1*H*,5*H*-pyrido[3,2,1-*ij*]quinoline).

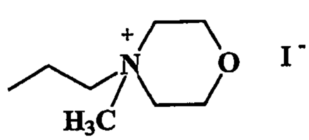
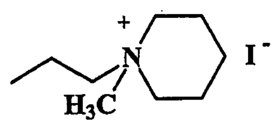
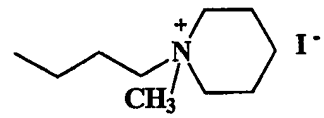
According to the invention, the preferred products are those in which the group $(X)_pR_2$ contains substituents bearing tertiary or quaternary nitrogen. In particular, the said group $(X)_pR_2$ is preferably represented by:

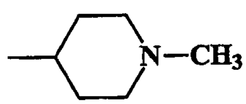
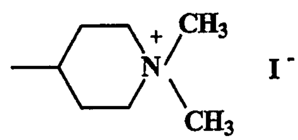
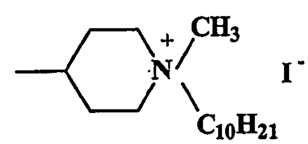


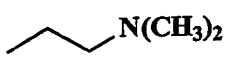
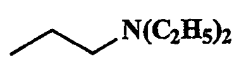


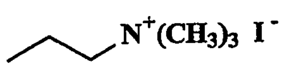
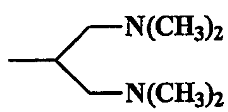
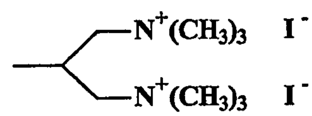


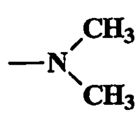
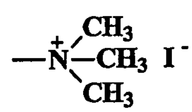




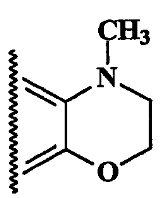
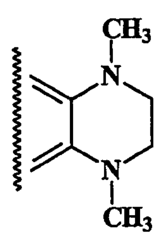
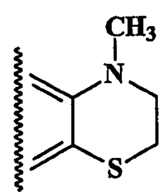




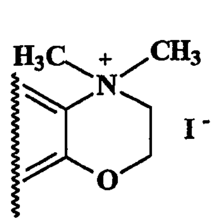
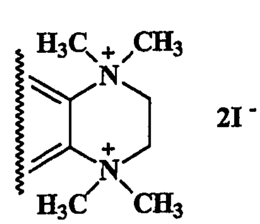
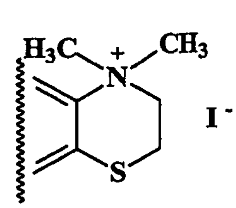




The compound in the present invention can be prepared in homogeneous as well heterogeneous phase, according to synthesis known in organic chemistry (and also described in the above cited patent) as well as by using the sub-phthalocyanine route.

Cationic phthalocyanines of formula (I) can be prepared by reacting the corresponding neutral compounds previously described, with an excess of alkyl iodide, with or without organic solvents, at temperature comprised between room temperature and reflux, for a time comprised between 1 h and 200 h. Pharmaceutically acceptable salts of the phthalocyanine compounds of the present invention, bearing basic substituents, include conventional acid addition salts, obtained by the addition of HCl, H_3PO_4, H_2SO_4, HBr, etc.

Additionally, salts obtained by reaction from the carboxylic function or acid groups within the phthalocyanine ring are within the scope of the present invention. Such salts include, for example, salts of carboxylic and sulfonic acid with amine derivatives, basic aminoacids and inorganic bases.

The present compounds possess both value as PDT agents themselves and also allows for their binding to carrier structures able to recognise biological targets involved in diseases, thus for the preparation of target specific derivatives. The compounds having formula (I) have utility as PDT dyes for treatment of infectious diseases of viral, fungine and bacterial origin, for use in cancer therapy and for dermatological diseases, moreover they may have applications as diagnostic aid in localising pathologically affected areas.

By conventional chemistry, the mono-carboxy phthalocyanines are convertible to a wide range of aliphatic and aromatic esters or amides, bearing one or more substituents on the alkyl or aromatic groups. Derivatives include esters, amides, amino acids, peptides, proteins (in particular antibodies), sugars, aptamers, sulfonic acid esters, etc. Phthalocyanines polyhydroxylated derivatives, such as glycosides compounds containing mono or polysaccharides, are of great utility as PDT agents, since the resultant derivatives are hydrophilic in contrast to the more hydrophobic phthalocyanines lacking such substituents. As hydrophobic and hydrophilic phthalocyanines concentrate selectively at different sites in a cellular environment, they may have useful applications.

By conventional chemistry the amino phthalocyanines are converted to alkyl, alicyclic, arilalkyl or aromatic secondary and tertiary amines or to amides. The primary amino substituent may also be converted to a diazonium salt and, by subsequent displacement reactions, halo and related derivatives are obtained. The amino group of mono amino substituted phthalocyanine also facilitates easy linkage of the phthalocyanine ring to peptides and proteins with the accompanying transport and binding benefits described above.

Of particular interest are the phthalocyanine derivatives in which amino groups not involved in the linkage with the carrier are further converted to quaternary ammonium salts with various alkylating agents, since for these compound selective activity against Gram -/+ or yeasts micro-organism have been found.

By using solid phase organic synthesis strategies, it is possible to bind the phthalocyanine moiety to a pre-assembled side chain protected polypeptide or polynucleotide attached to a solid phase, allowing for a specific binding of phthalocyanine to the N-terminal of peptide or nucleotide with no disturbance to its structure, thus improving the overall recognition of the target.

As it can be seen from formula (I), the phthalocyanine derivatives object of this invention are aromatic compounds exhibiting improved absorption and singlet oxygen photosensitization characteristics. These compounds contain substituents able to improve their photosensitizing properties and/or to cause a red shift in their light absorption, while retaining the photodynamic characteristics. These compounds have a side chain capable of attachment to predetermined functional groups, which serves as an handle for attachment to protein or to others carriers which may recognize a specific target on a biological structure.

Either the substituents introduced or the conjugation of the phthalocyanines with proteins may accelerate metabolism of the phthalocyanine moiety by interaction with light, thus destroying *in vivo* the light absorbing chromophore, in order to generate non-photoactivatable metabolites which are photochemically innocuous and thus unable to cause post-PDT phototoxicity.

For example, the presence of hydrophilic substituents and the conjugation is able to accelerate the elimination of those molecules that have not reached the *in vivo* target. The absorbing chromophore can be eliminated *in vivo,* thus avoiding the

insurgence of delayed cutaneous or systemic toxicity, generating not-photoactivatable, not toxic photodecomposition products.

Derivatives such as quaternary ammonium salts or sulphonate salts are also important, since cationic and anionic dyes are able to concentrate in different areas of cells. Phthalocyanines covalently bound through peptide linkages to peptides or proteins provide PDT agents with valuable specific transport and selective binding characteristics.

Compounds of the present invention are therefore superior to simple derivatized phthalocyanines, with regard to rapid clearance from the body after administration. They are also superior with regards to the toxicity after conjugation to specific carriers, because of the minor dosage due to the specific localization in the ill area.

A major improvement of the molecules object of the present invention is the red shifted absorption they have. Red light of wavelength higher than 670 nm is very much suitable for safe treatment of various diseases. Since light of wavelength lower than 650 nm loses most of its energy after penetrating into human tissues, higher wavelengths are more appropriate than short-penetrating lower wavelengths for dye activation, in applications such as tumours and infectious diseases not superficially located.

The conjugation to macromolecules provides a way to further increase the maximum wavelength absorbance.

Compounds which can be used for conjugation with the phthalocyanines according to the invention are for example: amino acids, peptides, proteins, antibodies, glycosides, aptamers; for example: Avidine, Concanavalin A, Succinil Concanavalin A, Monoclonal and recombinant Antibodies or fragments thereof etc.

Further, since the transport, mobility, binding to cell receptors is matter related to chemical structure and, in particular, to the hydrophilic or hydrophobic character of the dye, it is clearly beneficial to have an available primary chemical structure which can be subject to extensive chemical structural manipulation.

When the compound contains a linked amino acid, a peptide or a protein, they are generally bonded to the compounds by means of an amide, thioether, disulfide or an ester linkage. For example, an amino acid may be bound through a carboxyl

group on the phthalocyanine, by means of the alpha-amino or other amino group present in the amino acid to form an amide linkage, or the amino group on the phthalocyanine can be bound to the carboxyl group present on the amino acid.

Suitable amino acids include the 20 naturally occurring amino acids in both the R and S forms, as well as non naturally occurring synthetic amino acids.

Peptides may be similarly bound to the ring structure of phthalocyanine and generally contain 2-20 amino acids, although a complete protein (especially the ones showing specificity for a target) may be used as carriers.

The phthalocyanine may be linked to proteins by the above mentioned carboxyl or amino groups or by using other specific functional groups as thiols, maleimide derivatives, alpha bromo esters and amides, diazonium salts and azide derivatives.

In the phthalocyanine glycosides derivatives, the sugar moiety, which may consists of a single sugar, either in the open or cyclic form, an oligosaccharide or a polysaccharide, may be attached to the phthalocyanine ring system by means of a conventional glycoside bond. Any of the common monosaccharide sugars and oligosaccharides thereof may be used to prepare the phthalocyanine glycosides of the present invention.

By using closely related conventional chemistry, conjugates constituted by the phthalocyanine derivatives described and aptamers can be prepared.

All of the many possible derivatives embrace the intact macrocyclic phtalocyanine chromophore and all are capable of generating singlet oxygen or radicals under appropriate irradiation conditions, each constituting therefore a prospective photoactivatable dye for use in PDT.

Using the above described procedure, the following products were obtained; in particular in Examples 1 – 15 amino reactive compounds (i.e. compounds wherein R is a group capable of reacting with an amino-group) are described, in Example 16, R is a group capable of reacting with Tyr and His, in Examples 17-19 biotine functionalised derivative are described, in Examples 20-21 R, is a group capable of reacting with a thiol-group and in Example 23, R is a group capable of reacting with a carbohydrate. In Example 23, R is a group capable of forming ester linkages with carboxyl groups.

Example 1

2-[(4-hydroxycarbonyl)phenoxy]-{[9,10][16,17][23,24]-tribenzo}zinc(II)phthalo cyanine. $C_{51}H_{26}N_8O_3Zn$; green-blue solid; UV-vis (DMF) λ_{max} 746, 725, 339; ESI-MS, *m/z* 863 $[M+H]^+$.

Example 2

2-[(4-hydroxycarbonyl)phenoxy]-9(10),16(17),23(24)-tri[2-(morfolin-1-yl)ethoxy] zinc(II) phthalocyanine. $C_{57}H_{53}N_{11}O_9Zn$; green-blue solid; UV-vis (DMF) λ_{max} 678, 611, 358, 276; ^{1}H-NMR ($DMSOd_6$), δ 9.5-9.3 (m, 1H), 9.3-9.0 (m, 4H), 9.0-8.8 (m, 3H), 8.2-8.0 (m, 2H), 7.8-7.6 (m, 4H), 7.5-7.3 (m, 2H), 4.8-4.5 (m, 6H), 3.85-3.65 (m, 12H), 3.1-2.9 (m, 6H), 2.8-2.6 (m, 12H); ESI-MS *m/z* 1100.6 $[M+H]^+$, 987.6 $[M+C_6H_{13}NO]^+$.

Example 3

2-[(4-hydroxycarbonyl)phenoxy]-2(3),9(10),16(17),23(24)-tri[2-(piperidin-1-yl) ethoxy] zinc(II)phthalocyanine. $C_{60}H_{59}N_{11}O_6Zn$; UV-vis (DMF) λ_{max}, (ε, M^{-1} cm^{-1}) 678 (1.308 x 10^5), 612, 355; ^{1}H NMR ($DMSO-d_6$) δ 9.55-8.60 (m, 10 H), 8.00-7.55 (m, 6 H), 4.95-4.35 (m, 6 H), 3.10-2.80 (m, 6 H), 2.80-2.35 (m, 12 H), 1.85-1.35 (m, 18 H) ; ESI-MS *m/z* 1094.7 $[M+H]^+$.

Example 4

2-[(4-hydroxycarbonyl)phenoxy]-1(4),8(11),15(18),22(25)-tri[2-(morfolin-1-yl) ethoxy] zinc(II)phthalocyanine. $C_{57}H_{53}N_{11}O_9Zn$; green-blue solid; UV-vis (DMF) λ_{max} 762, 691, 623, 340, 268, 259; ^{1}H-NMR ($DMSOd_6$) δ 9.5-8.6 (m, 4H), 8.3-7.1 (m, 12H), 5.2-5.0 (m, 2H), 5.0-4.75 (m, 4H), 3.75-3.65 (m, 8H), 3.65-3.5 (m, 4H), 3.3-3.15 (m, 2H), 3.0-22.85 (m,8H), 2.8-2.7 (m, 4H); FAB-MS *m/z* 1101 $[M+H]^+$, 987 $[M+C_6H_{13}NO]^+$.

Example 5

2-[(4-hydroxycarbonyl)phenoxy]-9(10),16(17),23(24)-tri[3-(dimethylamino)phenoxy] zinc(II)phthalocyanine. $C_{63}H_{47}N_{11}O_6Zn$; green-blue solid; UV-vis (DMF) λ_{max} (ε, M^{-1} cm^{-1}) 678 (1.4680 x 10^5), 632, 611, 355; ^{1}H NMR ($DMSO-d_6$) δ 9.35-8.90 (m, 4 H), 8.85-8.50 (m, 3 H), 7.95-7.48 (m, 7 H), 7.52-7.25 (m, 5 H), 6.95-6.55 (m, 9 H), 3.10-2.80 (m, 18 H) ; FAB-MS *m/z* 1118 $[M+H]^+$.

Example 6

2-[(4-hydroxycarbonyl)phenoxy]-8(11),15(18),22(25)-tri[3-(dimethylamino)phenoxy]

zinc(II)phthalocyanine. $C_{63}H_{47}N_{11}O_6Zn$; green-blue solid; UV-vis (DMF) λ_{max} (ε, M^{-1} cm^{-1}) 689 (1.5064 x 10^5), 620, 333; ^1H NMR (DMSO-d_6) δ 9.50-8.70 (m, 6 H), 8.68-7.72 (m, 6H), 7.58-6.95 (m, 5H), 6.85-6.45 (m, 9 H), 6.40-6.30 (m, 2 H), 3.10-2.79 (m, 18 H) ; FAB-MS *m/z* 1118 $[M+H]^+$.

Example 7

2-[(4-hydroxycarbonyl)phenoxy]-9(10),16(17),23(24)-tri[3-(trimethylammmonium) phenoxy]zinc(II)phthalocyanine triiodide. $C_{66}H_{56}I_3N_{11}O_6Zn$; green-blue solid.

Example 8

2-[(4-hydroxycarbonyl)phenoxy]-8(11),15(18),22(25)-tri[3-(trimethylammmonium) phenoxy] zinc(II) phthalocyanine triiodide. $C_{66}H_{56}I_3N_{11}O_6Zn$; green-blue solid; UV-vis (DMF) λ_{max} 689, 620, 333 ; ESI-MS *m/z* 388 $[M-3I^-]^{3+}$.

Example 9

2-[(4-hydroxycarbonyl)phenoxy] zinc(II)phthalocyanine. $C_{39}H_{21}N_8O_3Zn$; blue solid; UV-vis (DMF) λ_{max} 669, 606, 343 ; ^1H NMR (DMSO-d_6) δ 9.32-9.14 (m, 7 H), 8.77 (broad s, 1 H), 8.26-8.18 (m, 8 H), 7.96-7.90 (dd, 1 H, J_1 = 8.67 Hz, J_2 = 1.73 Hz), 7.53 (d, 1 H, J = 8.59 Hz).

Example 10

2-[(4-hydroxycarbonyl)phenoxy]-9,10,16,17,23,24-esa[3-(dimethylamino)phenoxy] zinc(II)phthalocyanine. $C_{87}H_{74}N_{14}O_9Zn$; green-blue solid; FAB-MS *m/z* 1524 $[M^+]$.

Example 11

2-[(4-hydroxycarbonyl)phenoxy]-9,10,16,17,23,24-esa[3-(trimethylammonium) phenoxy] zinc(II)phthalocyanine esaiodide. $C_{93}H_{92}I_6N_{14}O_9Zn$; green-blue solid.

Example 12

2-[(4-sulphonate)phenoxy]-9,10,16,17,23,24-esa[3-(dimethylamino)phenoxy] zinc(II) phthalocyanine. $C_{86}H_{74}N_{14}O_{10}SZn$; green-blue solid; ESI-MS *m/z* 1562 [$(M+H)^+$].

Example 13

2-[(4-sulphonate)phenoxy]-9,10,16,17,23,24-esa[3-(trimethylammonium)phenoxy] zinc(II)phthalocyanine esaiodide. $C_{92}H_{92}I_6N_{14}O_{10}SZn$; green-blue solid.

Example 14

2-[(4-hydroxycarbonyl)phenoxy]-9,10,16,17,23,24-esa[2-(*N,N*-diethylamino) ethylthio] zinc(II)phthalocyanine. $C_{75}H_{98}N_{14}O_3S_6Zn$; green-blue solid.

Example 15

2-[(4-hydroxycarbonyl)phenoxy]-9,10,16,17,23,24-esa[2-(*N,N,N*-triethylammonium) ethylthio]zinc(II)phthalocyanine esaiodide. $C_{81}H_{116}I_6N_{14}O_3S_6Zn$; green-blue solid.

Example 16

2-[(4-aminobenzamidyl)-phenoxy]-8(11),15(18),22(25)-tri[3-(trimethylammmonium) phenoxy] zinc(II) phthalocyanine triiodide

λ_{max} 668, 607, 345, Blue crystals

Example 17

N,N'-dimethyl-*N*-2-(4-oxybenzoyl)-8(11),15(18),22(25)-tri[3-(trimethylammmonium) phenoxy] zinc(II) phthalocyanine *N'*-biotinyl -1,2-diaminoethane triiodide; λ_{max} 669, 605, 344, Deep blue crystals.

Example 18

N,N'-dimethyl-*N*-2-(4-oxybenzoyl) zinc(II) phthalocyanine *N'*-biotinyl-1,2-diaminoethane. Green-blue solid.

Example 19

N,N'-dimethyl-*N*-2-(4-oxybenzoxyl)-9,10,16,17,23,24-esa[3-(trimethylammonium) phenoxy] zinc(II) phthalocyanine *N'*-biotinyl-1,2-diaminoethane esaiodide. Green-blue solid.

Example 20

2-[(4-bromomethylcarbonyl)-phenoxy]-8(11),15(18),22(25)-tri[3-(trimethylammmonium) phenoxy] zinc(II) phthalocyanine triiodide

Example 21

2-[(4-maleimidomethyl)-phenoxy]-8(11),15(18),22(25)-tri[3-(trimethylammmonium) phenoxy] zinc(II) phthalocyanine triiodide

Example 22

2-[(4-hydrazidomethyl)-phenoxy]-8(11),15(18),22(25)-tri[3-(trimethylammmonium) phenoxy]zinc(II)phthalocyanine triiodide; λ_{max} 669, 605, 344, Blue crystals.

Example 23

2-[(2-hydroxy)ethyoxy] zinc(II) phthalocyanine. $C_{34}H_{20}N_8O_2Zn$, green-blue solid.

CONJUGATES

Example 24

Compound of Ex.9-Bovine Serum Albumin (BSA)

12.5 and 25 equivalents of the succinimidyl ester of compound according to Ex. 9 (previously prepared by reacting the corresponding asymmetric anhydride with N-hydroxysuccinimido esters) as DMSO solution, are slowly added to 200 µl of a 5 mg/ml solution of bovine serum albumin (BSA) in PBS (pH = 8.5) maintaining the obtained suspension under gentle stirring at room temperature for 90 minutes.

The green-blue conjugation product is purified from the solution by gel filtration (Sephadex G25) eluting with PBS (pH = 7.2), collecting fractions having a volume of ca. 1 ml. The labelling ratio has been determined spectrophotometrically measuring the protein concentration and the number of moles of the compound of Ex. 9 per mole of BSA. In the practiced experimental conditions the labelling ratio resulted comprised between 4.2 and 5.0.

Compound of Ex. 7-Avidine

100 µl of a 1.54 mg/ml solution of the succinimidyl ester of compound of Ex. 7 in DMSO are added to 2 mg of avidine (4mg/ml in 100 mM PBS pH = 8.5). The obtained suspension is gently stirred for 12 hours at 4 °C, then centrifuged. A purification step is carried out by gel filtration (Sephadex G25) eluting with 100 mM PBS (pH = 8.4), collecting the coloured fractions, from which the conjugation product is recovered. The labelling ratio, determined as reported in the preceding Example.16 was 7.

Analogously the following conjugates were prepared:

Compound of Ex. 8-Concanavalin A

100 µl of a solution of the succinimidyl esther of the compound of Ex. 8, 1.5 mg/ml in DMSO are slowly added to 2 mg of concanavalin A (Sigma) solubilized in 0.25 ml of 100 mM phosphate buffer (pH: 8). The obtained suspension is gently stirred for one night at 4°C in the dark. After centrifugation the supernatant is purified by gel filtration on Sephadex G25 collecting the fractions showing a characteristic fluorescence. The conjugate has been characterised in terms of moles of phthalocyanine per mole of protein, value turned out to range between 3 and 7

Compound of Ex. 8-Succinil concanavalin A

The procedure is as reported for Concanavalin A. The ratio was found to range from 3 to 5.

Compound of Ex. 4- Antibody

Monoclonal Antibody α-D specific for the repeat D of type III FN like of human Tenascin (TN) (Balza et al. FEBS, 332,39 1993) has been labelled by using the compound of Ex. 4.

The labelling procedure has been carried out with the monoclonal bound to the Sepharose 4b immobilised antigen, represented from the recombinant TN containing repeats B,C,D type III FN-like. The labelling ratio Mab/Compound of Ex. 4 was found to be 1:5.

After labelling the binding specificity of the labelled antibody was determined by immunohystochemistry on human fibroblasts (GM6114) and found to be the same of the unlabelled one. Compound of Ex. 4 and the labelling procedure used does not cause aggregation to occur or protein denaturation as demonstrated by the above immuno hystochemistry experiments and can therefore be used to produce photoactives immunoconjugates.

Compound of Ex 7 peptides labelling on solid phase

Peptides were assembled using FMOC chemistry and various resins suitable for this type of synthesis. The compound of Ex. 7 was coupled to the N terminus of the peptide, branched peptides or loligomers by first activating its carboxylic group with 0.5 molar equiv. of dicycloexylcarbodiimide in DMF overnight at room temperature or alternatively after having formed a mixed anhydride or a succinimidyl esther. The activated compound (5 times molar excess to the N terminal amino groups) was added to the peptide resin in the dark and left to proceed for 24 h at room temperature. The Compound of Ex.7-peptides were cleaved from the resin, worked up by using the solid phase synthesis standard procedures and desalted by using G10 or G25 according to the need and PBS pH: 7.2. The conjugated were analyzed either by mass spectrometry and by their amino acid composition and found consistent with the expected figures.

Compound of Ex. 12 peptides labelling in solution

Compound of Ex. 12 (thiol directed phthalocyanine derivative) was solubilized in DMF and directly added in the dark to a solution of cysteine elongated: peptides, branched peptides and loligomers, in a degassed, nitrogen flushed PBS pH 8.1 solutions. The reaction was left to occur for 24 h at room temperature, then the

Compound of Ex.12-peptides were desalted by using G10 or G25 according to the need and PBS pH 7.2. The conjugated were analysed either by mass spectrometry and by their amino acid composition and found consistent with the expected figures.

BIOCIDAL ACTIVITY

The lack of non specific toxicity has been assessed by using human fibroblasts cultured for six days. Aliquots of the compound according to Ex. 4 at various concentrations have been added to cells in DMEM 10% FCS. After treatment the cell were irradiated for 10 min with red light (Intralux 4000 equipped with filter BP700/100Chroma Technology Corp.). In a parallel experiment cells were treated with the same amounts of compound of Ex. 4, however no light was provided. No differences in mortality or morphology was detected in comparison to not treated-not irradiated cells up to a 40 μM compound of Ex. 4 concentration.

In a second experiment, not related cell still in DMEM 10% FCS were treated with several aliquots of mAb compound of Ex. 4 conjugated at an equivalent compound of Ex. 4 concentration up to 40 μM. After incubation, the cells were finally exposed for 10 min. to red light (Intralux 4000 equipped with filter BP700/100 Chroma Technology Corp.) and the viability and morphology compared with treated but not irradiated and with not treated, not irradiated cells. No differences were noticed.

Those experiments demonstrate that the compound of Ex. 4 by itself or as mAb conjugated is not toxic for fibroblasts or toward not related cells, well above the standard concentration used to inactivate living form by using PDT.

The usefulness of the compound of the present invention is further demonstrated through their activity against a variety of micro-organisms. The following example refer to the activity against *C. albicans*.

Fig. 1 shows the photoinactivation of *C. albicans* by the compounds according to Ex. 7 and 8 (indicated in the figure with MRLP 090 and MRLP 091 respectively).

THERAPEUTIC FORMULATIONS

Compounds as previously described may be used either for topical treatment of superficial diseases or after parenteral administration.

Therapeutic compositions containing the compounds of the present invention include liposome or microvesicle preparations, dispersions, ointments, solutions

for parenteral injection, etc. and include topical dermatological preparations.

Parenteral Solutions

The photoactivatable phthalocyanines generally are used with additional solvents and adjuvants to prepare solutions suitable for intravenous injection. A number of solvents and co-solvents, that are miscible with water and suitable surfactants, can be used to achieve solutions for parenteral use. The most important solvents in this group are ethanol, polyethylene glycols of the liquid series and propylene glycol. A more comprehensive listing includes dimethyl sulfoxide, ethanol, glycerin, polyethylene glycol 300 and 400, propylene glycol, sorbitol, polyoxyethylene sorbitan fatty acid esters such as laurate, palmitate, stearate, and oleate, polyoxyethylated vegetable oil, sorbitan monopalmitate, 2-pyrrolidone, N-methylpyrrolidine, N-ethylpyrrolidine and tetrahydrofurfuryl alcohol.

Other additives may be necessary to enhance or maintain chemical stability and physiological suitability. Examples are antioxidants, chelating agents, inert gases, buffers and isotonicifiers .

Topical Formulations

The phthalocyanine compounds of the present invention may be formulated for topical application in penetrating solvents or in the form of a lotion, cream, ointment or gel containing a sufficient amount of the phthalocyanine compound to be effective for PDT.

Suitable penetrating solvents are those which will enhance percutaneous penetration of the phthalocyanine compound. Solvents having this property include dimethyl sulfoxide, 1-methyl-2-pyrrolidone, azone and propylene glycol. DMSO solutions containing 0-50 wt.% water are particularly desirable.

Liposome or Microvesicle Preparations

Liposomes are microvesicles which encapsulate a liquid within lipid or polymeric membranes; the methods of preparing liposomes for both topical and parenteral (injectable) preparations are known in the art. The phthalocyanine compounds of the present invention having lipophilic characteristic may be incorporated into liposome microvesicles and used in this form for both topical and parenteral application.

Photodynamic therapy using the phthalocyanine compounds of the present

invention has a number of advantages. The phthalocyanine compounds itself are minimally toxic in the unexcited state. Each phthalocyanine molecule can be repeatedly photoactivated and leads each time to cell-lethal events, that is the generation of singlet molecular oxygen or radicals. The half-life of singlet oxygen is such that the target cell is affected without the opportunity for migration of the lethal singlet oxygen to neighbouring healthy tissue cells. Singlet oxygen molecules rupture chemical bonds in the cell DNA, target cell wall, or destroy intracellular structures such as mitochondria, resulting in destruction of the target cell. Destruction of target cell tissue commences promptly upon irradiation of the phthalocyanine compounds and ceases abruptly when irradiation is stopped. Photodynamic therapy using the compounds of the present invention is therefore selective and minimally toxic to healthy tissue. Singlet oxygen molecules produced which do not react rapidly with neighboring molecules rapidly decay.

A variety of phototherapy and irradiation methodologies are known to those skilled in the art and can be used with the novel phthalocyanine compounds of the present invention. The time and duration of therapy and repetition of the irradiation treatment can be selected by the physician according to known photodynamic therapy criteria. The dosage of the phthalocyanine compound may be varied according to the size and location of the target tissues which are to be destroyed and the method of administration. Generally, the dosage will be in the range of 0.1-20 mg of phthalocyanine compound per kilogram of body weight, more preferably in the range of 0.1 - 5.0 mg/kg.

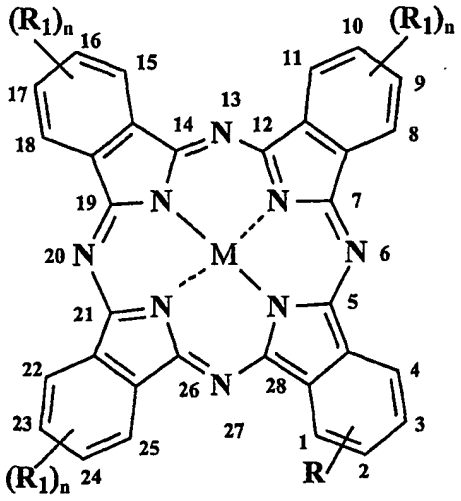
For cancer therapy ad treatment of infectious diseases, irradiation generally takes place not less than one hour and nor more than four days after administration of the phthalocyanine compound. Usually, phototherapy is begun approximately 10 hours to 24 hours after administration of the photodynamic therapy agent. For dermatological applications like psoriasis, but also for infectious diseases or cancer treatment, radiation therapy can commence immediately after topical application of the phthalocyanine or up to 12 hours later. Systemic application for treatment of dermatological diseases is followed by radiation usually 15 to 24 hours after systemic administration of the PDT agent. Exposure to non therapeutic light sources should be avoided immediately following phototherapy to minimise

light toxicity. Appropriate cover of the patient can be used, to limit the area affected by phototherapy.

Light sources appropriate for the use in PDT are well known in the art and may vary from white light sources associated with appropriate filters to lasers settled to the right wavelength. As noted above, preferred wavelengths are from 600 to 950 nm, preferably from about 650 to about 750 nm. The total amount of light which is applied to the affected area will vary with the treatment method used and with the location of the lesion. Generally, the amount of light is in the range of about 50 to 1000 Jcm^{-2}, preferably in the range of 100 to 350 Jcm^{-2}.

CLAIMS

1. Metal substituted non centrosimmetrical phthalocyanine of formula (I):



(I)

wherein :

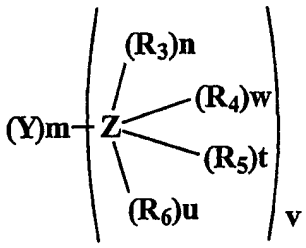
n is 1, 2 or 4;

M is chosen in the group consisting of Zn, $Si(OR_7)_2$ and $AlOR_7$ wherein R_7 is chosen in the group consisting of H, C_{1-15} alkyl and pharmaceutically acceptable salts thereof;

R is chosen in the groups consisting of: -COOH, -SH, -OH, $-NH_2$, $-CO-CH_2-Br$, $-SO_2Cl$, maleimide, hydrazide, phenol, imidate, biotine, possibly bound to the phthalocyanine nucleus through a suitable linker and

R_1 is H or, when n = 2 and the two groups R_1 are in the positions 9,10,16,17,23,24, said two groups R_1 can form a saturated or unsaturated heterocycle, possibly substituted, which may contain up to two heteroatoms chosen from N,O, S; or R_1 is represented by the group $(X)_pR_2$, wherein:

X is chosen in the group consisting of O, S, $-NR_5$ and $-CH_2-$ and R_2 is



where :

Y is chosen in the group consisting of C_{1-10} alkyl and phenyl, possibly substituted, or it forms with the Z group, to which it is bound, a saturated or unsaturated heterocycle, possibly substituted, which may contain up to two heteroatoms chosen in the group consisting of N, O and S;

Z is chosen in the group consisting of -N, $-CH_2N$ and $-CONHCH_2CH_2N$;

R_3 and R_4, equal or different from one another, are chosen in the group consisting of C_{1-15} alkyl and phenyl, or form with the Z group, to which they are bound, a saturated or unsaturated heterocycle, possibly substituted, which may contain up to two heteroatoms chosen in the group consisting of N, O and S;

R_5 and R_6, equal or different from one another, are chosen in the group consisting of H and C_{1-15} alkyl ;

m, n, p, w, t and u, independently from one another, are 0 or 1; and

v is an integer comprised between 1 and 3;

with the proviso that:

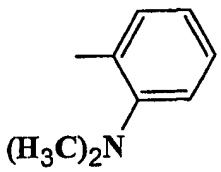
R is in the position 1 or 2.

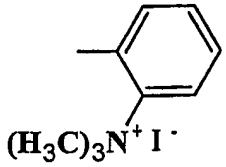
R_1 is in the positions: 8(11), 15(18), 22(25), or 9(10), 16(17), 23(24) when n = 1.

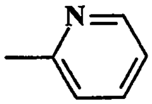
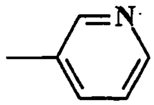
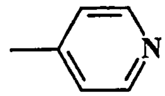
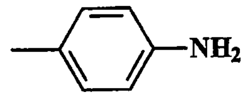
R_1 is in the positions: 8,11,15,18, 22,25 or 9,10,16,17,23,24 when n = 2.

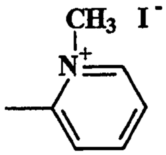
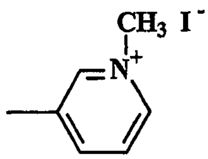
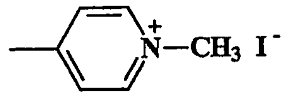
2. Metal phthalocyanine according to Claim 1 wherein M is Zn.

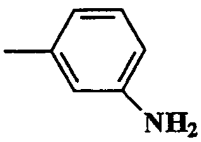
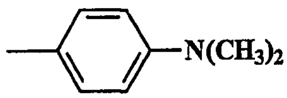
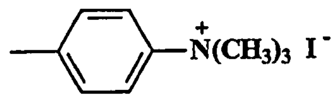
3. Metal phthalocyanine according to Claim 1 wherein the group $(X)_pR_2$ is chosen in the group consisting of:

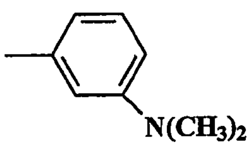
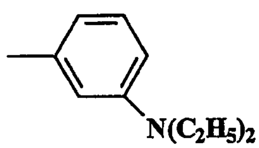
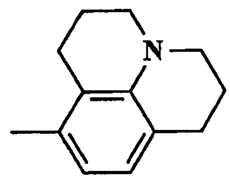


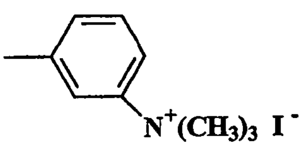
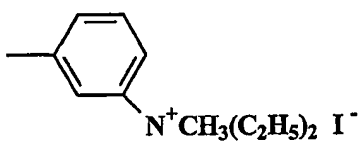
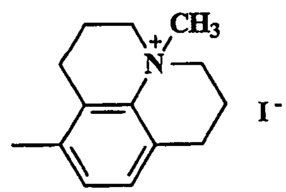


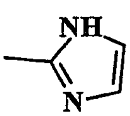
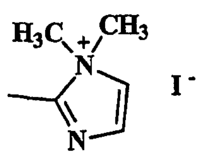
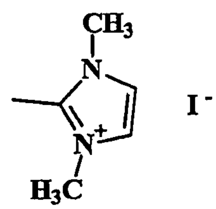





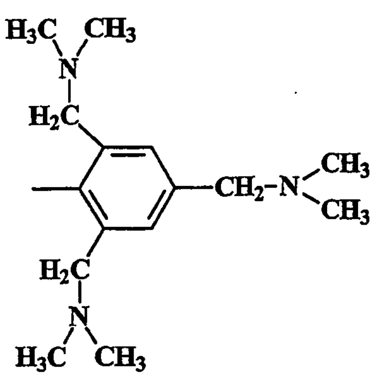
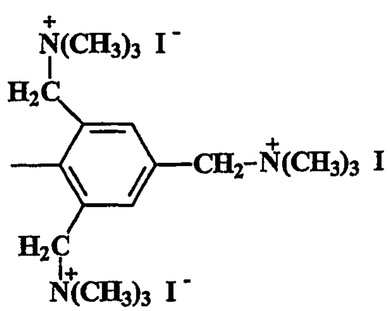




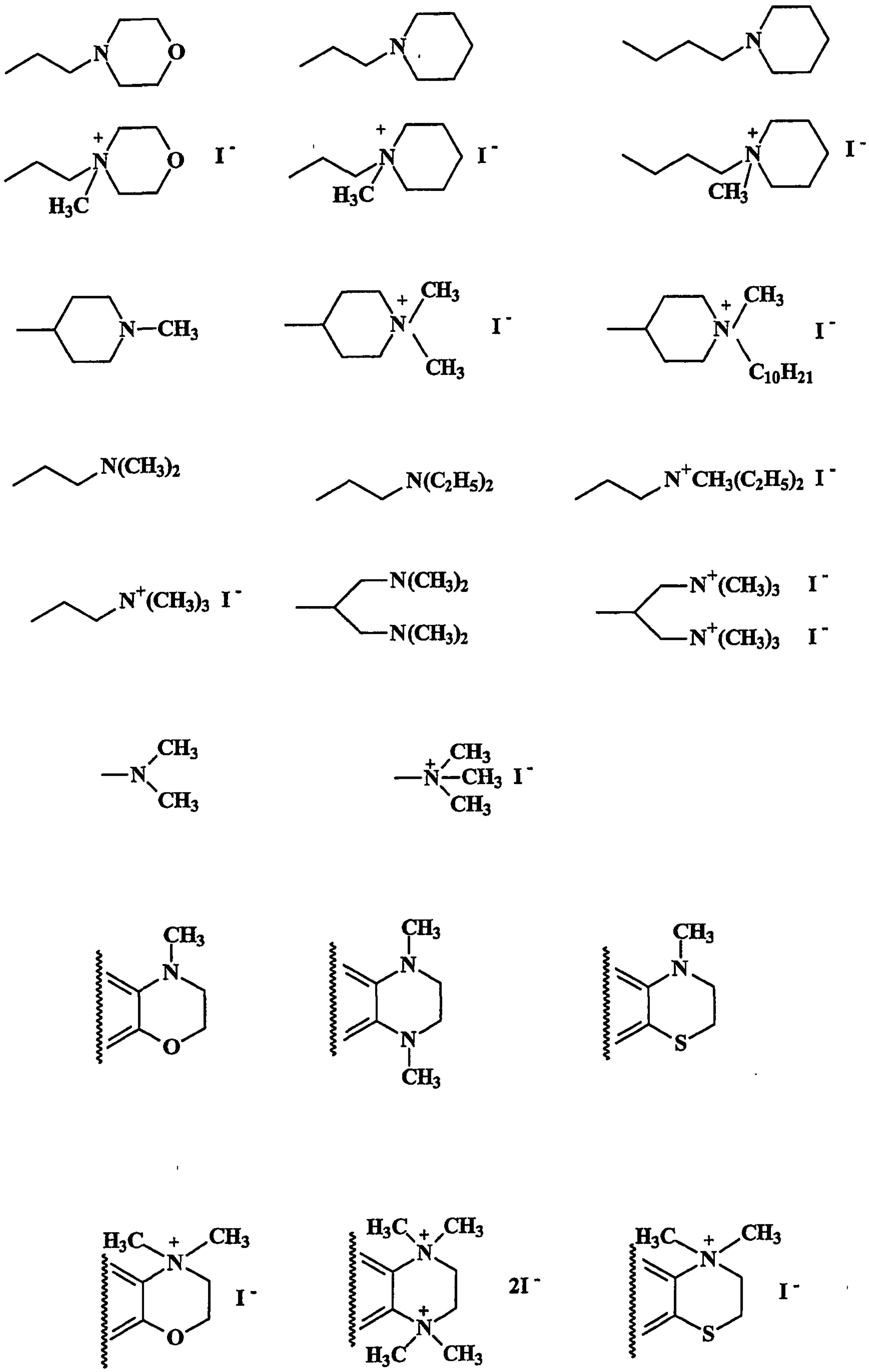






N
O
N
N
N
O
I⁻
H3C
N
I⁻
H3C
N
I⁻
CH3
N—CH3
CH3
N
I⁻
CH3
CH3
N
I⁻
C10H21
N(CH3)2
N(C2H5)2
N+CH3(C2H5)2 I⁻
N+(CH3)3 I⁻
N(CH3)2
N(CH3)2
N+(CH3)3 I⁻
N+(CH3)3 I⁻
CH3
N
CH3
CH3
N—CH3 I⁻
CH3
CH3
N
O
CH3
N
N
CH3
CH3
N
S
H3C
CH3
N
O
I⁻
H3C
CH3
N
N
H3C
CH3
2I⁻
H3C
CH3
N
S
I⁻

4. Metal phthalocyanine according to Claim 1 represented by:
2-[(4-hydroxycarbonyl)phenoxy]-{[9,10][16,17][23,24]-tribenzo}zinc(II)phthalo cyanine;
2-[(4-hydroxycarbonyl)phenoxy]-9(10),16(17),23(24)-tri[2-(morfolin-1-yl) ethoxy]zinc(II)phthalocyanine;
2-[(4-hydroxycarbonyl)phenoxy]-2(3),9(10),16(17),23(24)-tri[2-(piperidin-1-yl) ethoxy] zinc(II)phthalocyanine;
2-[(4-hydroxycarbonyl)phenoxy]-1(4),8(11),15(18),22(25)-tri[2-(morfolin-1-yl) ethoxy] zinc(II) phthalocyanine;
2-[(4-hydroxycarbonyl)phenoxy]-9(10),16(17),23(24)-tri[3-(dimethylamino)phenoxy] zinc(II)phthalocyanine;
2-[(4-hydroxycarbonyl)phenoxy]-8(11),15(18),22(25)-tri[3-(dimethylamino)phenoxy] zinc(II)phthalocyanine;
2-[(4-hydroxycarbonyl)phenoxy]-9(10),16(17),23(24)-tri[3-(trimethylammonium)-phenoxy] zinc(II)phthalocyanine triiodide;
2-[(4-hydroxycarbonyl)phenoxy]-8(11),15(18),22(25)-tri[3-(trimethylammmonium)-phenoxy] zinc(II)phthalocyanine triiodide;
2-[(4-hydroxycarbonyl)phenoxy]zinc(II)phthalocyanine;
2-[(4-aminobenzamidyl)-phenoxy]-8(11),15(18),22(25)-tri[3-(trimethylammmonium) phenoxy] zinc(II)phthalocyanine triiodide;
N,N'-dimethyl-N-2-(4-oxybenzoxyl)-8(11),15(18),22(25)-tri[3-(trimethyl-ammonium) phenoxy] zinc(II)phthalocyanine N'-biotinyl -1,2-diaminoethane triiodide;
2-[(4-bromomethylcarbonyl)-phenoxy]-8(11),15(18),22(25)-tri[3-(trimethylammonium)-phenoxy]zinc(II)phthalocyanine triiodide;
2-[(4-maleimidomethyl)-phenoxy]-8(11),15(18),22(25)-tri[3-(trimethylammonium)-phenoxy]zinc(II)phthalocyanine triiodide;
2-[(4-hydrazidomethyl)-phenoxy]-8(11),15(18),22(25)-tri[3-(trimethylammonium)-phenoxy]zinc(II)phthalocyanine triiodide;
2-[(4-hydroxycarbonyl)phenoxy]-9,10,16,17,23,24-esa[3-(dimethylamino)phenoxy] zinc(II)phthalocyanine;
2-[(4-hydroxycarbonyl)phenoxy]-9,10,16,17,23,24-esa[3-(trimethylammonium) phenoxy] zinc(II)phthalocyanine esaiodide;

2-[(4-sulphonate)phenoxy]-9,10,16,17,23,24-esa[3-(dimethylamino)phenoxy] zinc(II) phthalocyanine;

2-[(4-sulphonate)phenoxy]-9,10,16,17,23,24-esa[3-(trimethylammonium)phenoxy] zinc(II) phthalocyanine esaiodide;

2-[(4-hydroxycarbonyl)phenoxy]-9,10,16,17,23,24-esa[2-(*N,N*-diethylamino) ethylthio] zinc(II)phthalocyanine;

2-[(4-hydroxycarbonyl)phenoxy]-9,10,16,17,23,24-esa[2-(*N,N,N*-triethylammonium) ethylthio]zinc(II)phthalocyanine esaiodide;

N,N'-dimethyl-*N*-2-(4-oxybenzoyl) zinc(II) phthalocyanine *N'*-biotinyl-1,2-diaminoethane;

N,N'-dimethyl-*N*-2-(4-oxybenzoxyl)-9,10,16,17,23,24-esa[3-(trimethylammonium) phenoxy] zinc(II)phthalocyanine *N'*-biotinyl-1,2-diaminoethane esaiodide;

2-[(2-hydroxy)ethyoxy]zinc(II)phthalocyanine.

5. Conjugates consisting of a compound according to Claims 1 – 4 and a compound chosen from amino acids, peptides, proteins, antibodies, glycosides and aptamers.

6. Conjugates according to claim 5 represented by:

Compound of Ex.9-Bovine Serum Albumin (BSA)

Compound of Ex. 7-Avidine

Compound of Ex. 8-Concanavalin A

Compound of Ex. 8-Succinil Concanavalin A

Compound of Ex. 4-Monoclonal Antibody α-D.

7. Use of metal phthalocyanines and conjugates thereof according to Claim 1 – 6 for the preparation of pharmaceutical composition for photodynamic therapy.

8. Use according to Claim 7 wherein the pharmaceutical compositions are in the form useful for topical administration.

9. Use according to Claim 7 wherein the pharmaceutical compositions are in the form useful for parenteral administration.

10. Use of metal phthalocyanines and conjugates thereof according to Claims 1 – 6 for blood and blood derivatives sterilisation.

11. Use of metal phthalocyanines and conjugates thereof according to Claims 1 – 6 as in vivo/in vitro diagnostics.

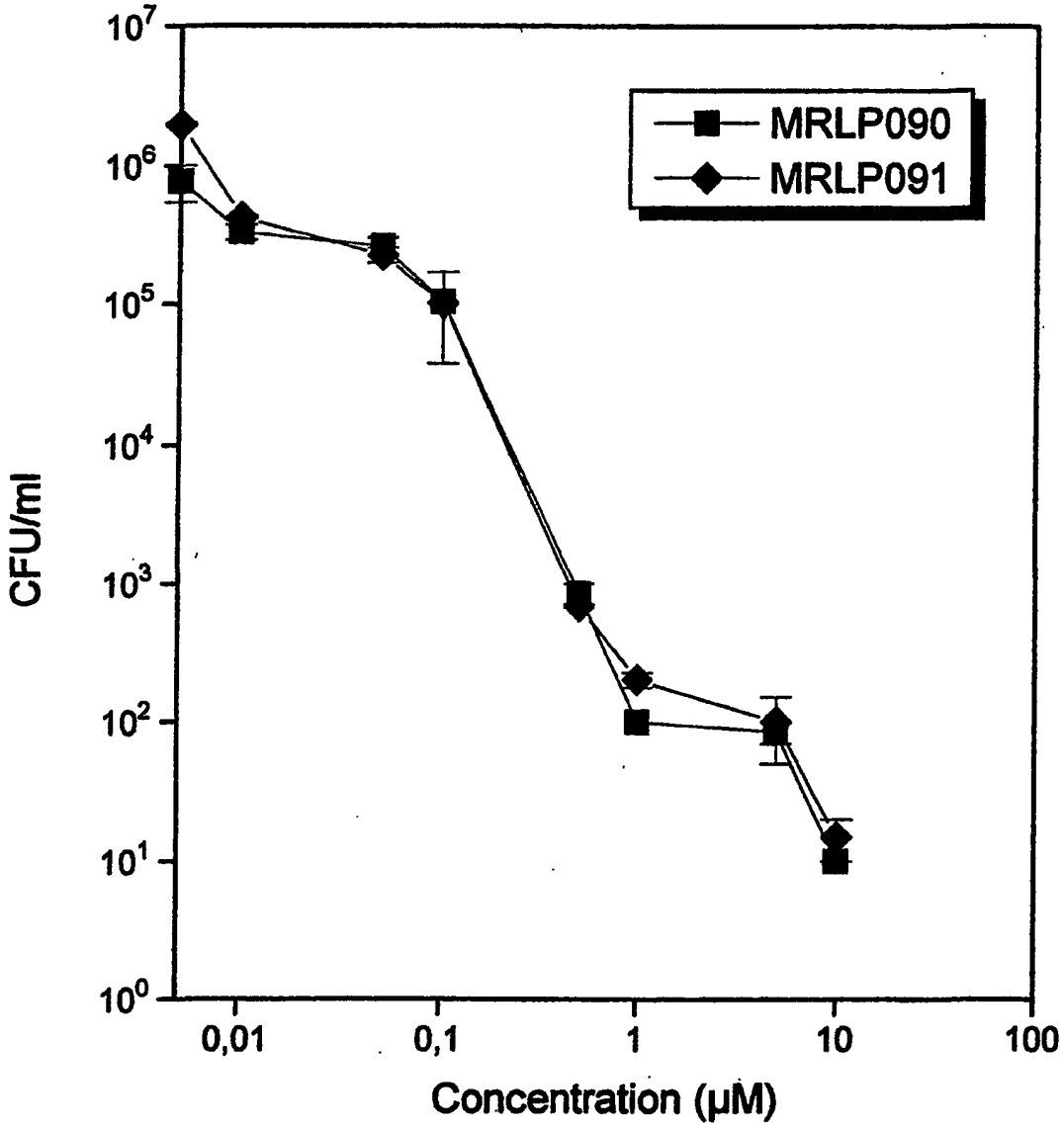

MRLP090
MRLP091
CFU/ml
10^7
10^6
10^5
10^4
10^3
10^2
10^1
10^0
0,01
0,1
1
10
100
Concentration (µM)

FIGURE 1

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 02/03108

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07D487/22 A61K31/555 A61K41/00 A61P43/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07D A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, PAJ, BEILSTEIN Data, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	SASTRE, A. ET AL.: "Synthesis of Novel Unsymmetrical Monoaminated Phthalocyanines" TETRAHEDRON LETTERS, ELSEVIER SCIENCE PUBLISHERS, AMSTERDAM, NL, vol. 36, no. 46, 13 November 1995 (1995-11-13), pages 8501-8504, XP004026948 ISSN: 0040-4039 Page 8501, last paragraph: "to bond them to biomolecules like monoclonal antibodies"; page 8502, compound 8; page 8503, last paragraph: "interesting candidates for photodynamic sensitization". --- -/--	1-11

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
14 August 2002	27/08/2002

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Weisbrod, T

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 02/03108

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	HU, M. ET AL.: "Hydroxyphtalocyanines as Potential Photodynamic Agents for Cancer Therapy" J. MED. CHEM., vol. 41, 1998, pages 1789-1802, XP002175080 Abstract; compounds 32, 34, 39, 45.	1-11
Y	HERTER, R. ET AL.: "Synthesis of NIR-Absorbing Monofunctionalized Phtalocyanines ..." PROC. SPIE-INT. SOC. OPT. ENG., vol. 2625, 1996, pages 384-385, XP001013015 Page 384, penultimate paragraph; page 385, paragraph 1, last sentence; and the zinc-phthalocyanine on page 385.	1-11
Y	SAVITSKII, A. P. ET AL.: "Avidin-Biotin System as an Agent for Targeted Delivery of Antitumor Agents" ROSS. KHIM. ZH., vol. 42, no. 5, 1998, pages 77-83, XP001008633 Page 81, scheme 2: monobiotinylated octacarboxy cobaltphtalocyanine and dibionylated trisulfo hydroxyaluminum-phtalocyanine. & CHEMICAL ABSTRACTS, vol. 132, no. 6, 2000 Columbus, Ohio, US; abstract no. 69211f, page 1150; Abstract.	1-11
A	LEZNOFF, C. C. ET AL.: "The synthesis of a soluble, unsymmetrical phthalocyanine on a polymer support" TETRAHEDRON LETTERS, ELSEVIER SCIENCE PUBLISHERS, AMSTERDAM, NL, vol. 23, no. 30, 1982, pages 3023-3026, XP002088333 ISSN: 0040-4039 Page 3025, compound 13.	1-11
Y	EP 0 906 758 A (MOLTENI L & C DEI FRATELLI ALI) 7 April 1999 (1999-04-07) cited in the application Abstract; claims 1,3,5,7-16; example 8.	1-11
P,X	EP 1 164 135 A (MOLTENI & C DEI FLII ALITTI SO) 19 December 2001 (2001-12-19) Claims 1,2,6,7,9-16; page 10 and claim 7: compound 17.	1-11

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 02/03108

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
L	REIMLINGER, H.: "Nomenklatur Organisch-Chemischer Verbindungen" 1998 , WALTER DE GRUYTER , BERLIN XP002175081 Pages 80-82, chapter 3.1.4 "Nomenklatur von Verbindungen mit Radikalzentren an charakteristischen Gruppen"; in particular page 82, last paragraph. -----	4

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-4 and 5-11 (all part)

   Phthalocyanines (I) wherein M is Zn and R is COOH, COCH2Br or imidate; and subject-matter referring to such compounds (I).

2. Claims: 1-3, 5, and 7-11 (all part)

   Phthalocyanines (I) wherein M is Zn and R is SH or SO2Cl; and subject-matter referring to such compounds (I).

3. Claims: 1-3, 5, and 7-11 (all part)

   Phthalocyanines (I) wherein M is Zn and R is OH directly bound to the position 1 of the phthalocyanine nucleus, and R1 is different from H; and subject-matter referring to such compounds (I).

4. Claims: 1-3, 5, and 7-11 (all part)

   Phthalocyanines (I) wherein M is Zn and R is OH bound to the position 1 of the phthalocyanine nucleus through a linker; and subject-matter referring to such compounds (I).

5. Claims: 1-3, 5, and 7-11 (all part)

   Phthalocyanines (I) wherein M is Zn and R is OH directly bound to the position 2 of the phthalocyanine nucleus, and R1 is different from H; and subject-matter referring to such compounds (I).

6. Claims: 1-4, 5, and 7-11 (all part)

   Phthalocyanines (I) wherein M is Zn and R is OH to the position 2 of the phthalocyanine nucleus through a linker; and subject-matter referring to such compounds (I).

7. Claims: 1-3, 5, and 7-11 (all part)

   Phthalocyanines (I) wherein M is Zn and R is NH2 in position 1; and subject-matter referring to such compounds (I).

8. Claims: 1-3, 5, and 7-11 (all part)

   Phthalocyanines (I) wherein M is Zn, R is NH2 directly bound

to the position 2 of the phthalocyanine nucleus, and R1 is different from H; and subject-matter referring to such compounds (I).

9. Claims: 1-4, 5, and 7-11 (all part)

Phthalocyanines (I) wherein M is Zn, R is NH2 bound to the position 2 of the phthalocyanine nucleus through a linker; and subject-matter referring to such compounds (I).

10. Claims: 1-4, 5, and 7-11 (all part)

Phthalocyanines (I) wherein M is Zn and R is maleimide, phenol or biotine; and subject-matter referring to such compounds (I).

11. Claims: 1-4, 5, and 7-11 (all part)

Phthalocyanines (I) wherein M is Zn and R is hydrazide; and subject-matter referring to such compounds (I).

12. Claims: 1, 3, 5, and 7-11 (all part)

Phthalocyanines (I) wherein M is Si(OR7)2; and subject-matter referring to such compounds (I).

13. Claims: 1, 3, 5, and 7-11 (all part)

Phthalocyanines (I) wherein M is AlOR7; and subject-matter referring to such compounds (I).

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 1 (part)

The scope of claim 1, in as far as pharmaceutical acceptable salts of R7 are concerned, is so unclear (Article 6 PCT) that a meaningful International Search is impossible with regard to this definition.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

national application No.
PCT/EP 02/03108

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: 1 (part)
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
see FURTHER INFORMATION sheet PCT/ISA/210

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☒ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/EP 02/03108

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 0906758 A	07-04-1999	IT MI971940 A1	15-02-1999
		EP 0906758 A1	07-04-1999
		US 5965598 A	12-10-1999
EP 1164135 A	19-12-2001	EP 1164135 A1	19-12-2001
		AU 7246301 A	24-12-2001
		WO 0196343 A1	20-12-2001

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(21) International Application Number: PCT/EP2002/003108

(22) International Filing Date: 20 March 2002 (20.03.2002)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
01106411.0 21 March 2001 (21.03.2001) EP

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(74) Agent: **GERVASI, Gemma**; Notarbartolo & Gervasi S.r.l., Corso di Porta Vittoria, 9, I-20122 Milan (IT).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*

(48) Date of publication of this corrected version:
21 May 2004

(15) Information about Correction:
see PCT Gazette No. 21/2004 of 21 May 2004, Section II

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: METAL SUBSTITUTED NON CENTROSIMMETRICAL PHTHALOCYANINE ANALOGUES, THEIR PREPARATION AND USE IN PHOTODYNAMIC THERAPY AND IN VIVO DIAGNOSTIC

(57) Abstract: Phthalocyanine analogues having an active group able to link the phthalocyanine to carriers molecules and phthalocyanine analogues as phthalocyanine-carrier conjugates showing enhanced photodynamic properties, red shifted absorption characteristic, all useful for photodynamic therapy, are described. Photosensitizers themselves or the photosensitizers-carrier conjugates are useful compounds either for treatment of various infectious diseases, the in vivo eradication of micro-organisms as well as diseases characterized by cellular hyperproliferation, in particular tumours psoriasis, actinic keratosis, atheromas, endoarterial hyperplasia and prostate hyperplasia. The above compounds can be also useful for blood and blood derivatives sterilization and as in vivo/vitro diagnostics.

WO 2002/090361 A1